

A hotspot for threatened Mediterranean odonates in the Seybouse River (Northeast Algeria): are IUCN population sizes drastically underestimated?

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Several odonate species are threatened in the Mediterranean basin and some of them show alarming decreasing trends. The distribution and population estimations provided by the IUCN are based on occasional field sampling or non-rigorous methodologies and could be erroneous and misleading. To obtain reliable estimations of the population size and distribution of three threatened species, *Calopteryx exul*, *Coenagrion mercuriale*, and *Gomphus lucasii*, we first conducted capture-mark-recapture in a natural population during one flight season, and second we carried out intensive sampling of adults, larvae and exuviae in the Seybouse watershed, Northeast Algeria. In addition, a revision of odonate occurrence and distribution in the watershed was done by pooling information collected over six years (2010–2015). Our results show that population estimations of the three species are much higher than what the IUCN presents; that is, 2208 individuals of *C. exul* (22.08% of the estimated global population), 1765 individuals of *C. mercuriale*, and 11,204 individuals of *Gomphus lucasii* (about 4.5 times as large as the estimated global population). Moreover, a total of 42 species were recorded in the study site, of which seven are new. The mean number of localities per species increased by a factor of 2.47, e.g. from six to 12 in *C. exul*, two to 12 in *Coenagrion mercuriale* and five to 14 in *Gomphus lucasii*. Our results suggest that the Seybouse watershed is one of the most important areas in North Africa and the Mediterranean basin for these three threatened species and requires particular attention and an urgent conservation plan to reduce anthropogenic effects and maintain populations.

Keywords: Odonata; *Calopteryx exul*; *Coenagrion mercuriale*; *Gomphus lucasii*; distribution; endemic; population status; capture-mark-recapture

Introduction

The Mediterranean Basin is one of the world's richest areas in terms of faunal and floral diversity, and is consequently considered as one of the 25 Global Biodiversity Hotspots (Medail & Quezel, 1997; Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000). The assessment of the odonatofauna of this region listed 165 odonates, with 19% of the species classed as

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threatened (3% Critically Endangered, 8% Endangered and 8% Vulnerable) (Riservato et al., 2009). The southern part of the Mediterranean basin, i.e. North Africa, harbors 83 species which represent 50.3% of the Mediterranean odonatofauna, of which 14.3% of zygopterans and 4.2% of anisopterans are endemic (Samraoui et al., 2010). Considering the scant knowledge of local odonates and the increasing anthropogenic pressure on natural habitats, there is an urgent need for reassessment of the distribution and status of local odonates.

In North Africa, the Seybouse watershed (Northeast Algeria) shows remarkable Odonata species composition and might be key for the survival of some threatened species in the coming decades. Although the number of species recorded so far is 35, three of them are threatened; either Endangered (*Calopteryx exul* and *Coenagrion mercuriale*) or Vulnerable (*Gomphus lucasii*) (Khelifa et al., 2011). The North African endemic *C. exul* had not been recorded for about a century before 2007 but recent records have shown that there is a flourishing population in the Seybouse watershed, the only one known in Algeria and probably the largest one (Khelifa, 2013; Khelifa et al., 2013). This species is listed as endangered and most known populations are probably extinct or on the verge of extinction (Boudot, 2010a).

Coenagrion mercuriale is globally listed as Near Threatened but locally classed as Endangered in North Africa with only nine localities recorded in Algeria (Ferreira et al., 2015; Mahdjoub et al., 2014). The gomphid *Gomphus lucasii* is also a North African endemic, occurring only in Algeria and Tunisia, with 13 populations lost in the last 100 years and three that probably still exist (Samraoui & Corbet, 2000). This dragonfly is listed as Vulnerable at the global, Mediterranean and North African scale. Unfortunately, most records of the three latter species are not informative of population size because they are limited to qualitative instead of quantitative data and encompass a single life stage (adult instead of larva or exuviae) and small geographic areas (specific sites instead of the watershed network). In addition, the population estimations provided by the IUCN are not based on rigorous methodologies and thus do not provide reliable estimates and prediction of future trends. Therefore, new assessments of population size are required especially for threatened species to obtain reliable estimations.

There are two reliable ways to estimate population size in odonates: one is regular exuviae collection (Raebel, Merckx, Riordan, Macdonald, & Thompson, 2010; Samways, McGeoch, & New, 2010) and the other is capture-mark-recapture (CMR) of adults (Cordero-Rivera & Stoks, 2008). The first one avoids killing or injuring sampled individuals and gives an estimate of the local population (e.g. pond, lake, river or stream stretch), indicating the number of larvae that survived up to emergence. The second one, on the other hand, usually encompasses a large proportion of the local population and a small part of emigrants that come from other sites. Unlike the first method, the second has rarely been used to estimate population size (e.g. Macagno, Boano, Palestini, Stassi, & Rolando, 2008; Watts, Saccheri, Kemp, & Thompson, 2007), probably because it is challenging and time consuming to capture and recapture individuals over a long period of time, particularly in anisopterans.

This study aims to (1) re-evaluate the species richness and distribution in the watershed, including adults, larvae and exuviae records; and (2) estimate population size of three threatened species (*Calopteryx exul*, *Coenagrion mercuriale*, and *Gomphus lucasii*) based on regular capture-mark-recapture of adults.

Material and methods

Study site

The Seybouse watershed is the third largest freshwater network in Algeria with an area of 6471 km². It is situated in the heart of Numidia (Northeast Algeria). The Seybouse river results from

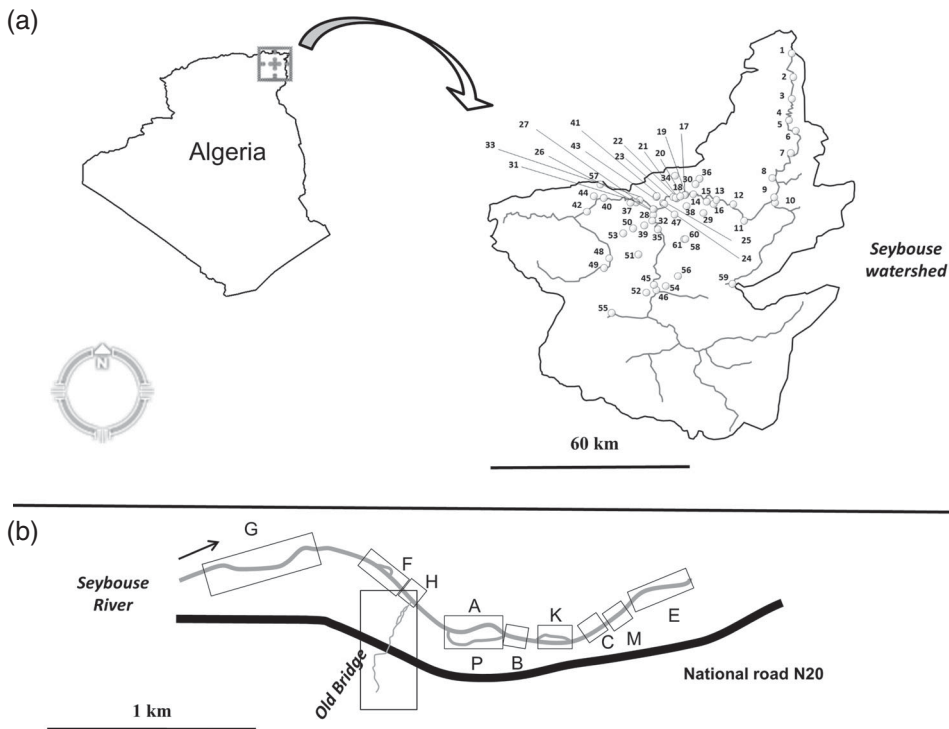


Figure 1. (a) Study localities sampled during the survey of the Seybouse watershed. The geographic coordinates and elevation of all localities are presented in Table S1. (b) Study sites where capture-mark-recapture surveys of *Coenagrion mercuriale*, *Calopteryx exul*, and *Gomphus lucasii* were carried out. The letters A, B, C, E, F, G, H, K, and M are the stretches where *C. exul* and *G. lucasii* were sampled (localities 19, 20, 21, 22). The non-sampled parts of the river were not accessible for sampling. *C. mercuriale* was surveyed in Old Bridge (locality 23).

the confluence of oued Bouhamdane and oued Cherf and flows in the Mediterranean in Sidi Salem ($36^{\circ}52'3''$ N, $7^{\circ}46'25''$ E) near Annaba. The hydroperiod is characterized by a wet season extending from October to May and a dry season spanning from June to September. The mean annual rainfall varies from 350 mm upstream to 608 mm downstream (ABHCSM, 2009).

Species richness and distribution

From 2010 to 2015, a total of 61 localities were sampled (Figure 1a, Table S1), of which 44 were visited only from May 2011 to 2015. Adults were sampled with a hand net along 100 m transect, most often in 47 localities ($N > 20$ visits) and occasionally in 14 additional localities ($N < 10$ visits). Exuviae were collected from the bank vegetation. Larvae were sampled regularly with a rectangular net (40×25 cm) from 2010 to 2015 in 15 localities in lotic water (localities 1, 2, 3, 4, 6, 8, 11, 14, 18, 22, 25, 32, 33, 45, 46) and from September 2013 to September 2014 in five lentic waterbodies (localities 5, 34, 41, 60, 61).

Capture-mark-recapture

In order to estimate population size of *Calopteryx exul*, *Coenagrion mercuriale*, and *Gomphus lucasii* daily CMR was carried out in two different sites (Figure 1b). *Calopteryx exul* and *Gomphus lucasii* were studied in 2 km stretch upstream on the Seybouse (localities 19–22), starting

from 9:30 to 16:00 in 2011. Six persons captured adults from the start of the flight season and marked them on the posterior wing with an alphanumeric code using permanent markers (Edding paint marker 780) (Figure 2a, b). During resightings, the code and sex of adults were recorded. In 2013, daily CMR of *Coenagrion mercuriale* was carried out by three persons from 9:30 to 16:00 in Old Bridge canal (locality 23) using the same CMR procedure as used on the two latter species (Figure 2c).

Population estimation

The Jolly–Seber model using POPAN formulation (Schwarz & Arnason, 1996) for abundance estimation was performed with the RMark package (Laake, 2013). The POPAN model includes four parameters: *Phi* (apparent survival), *p* (capture probability given the animal is alive and on the study area), *pent* (probability of entry into the population for this occasion), and *N* (super-population size). The model used to estimation abundance was *Phi* (time + sex) *p* (time + sex) *pent* (time + sex) *N* (sex), which allows survival, capture probability and probability of entry to vary by time and sex while the super-population size varies by sex. Sex ratio was estimated as the percentage of males. IM50, the time at which 50% of the number of adults estimated by the POPAN model had entered the population, was calculated in the three species to determine the temporal pattern of flight season.

Statistical analysis

Statistical analyses were conducted with the software R 3.1.2 (R Development Core Team, 2014). Chi-squared tests were performed to check if the sex ratio during the flight season deviates from equilibrium in the three study species. Skewness and D'Agostino skewness tests were computed with the R package *moments* (Komsta & Novomestky, 2015) to determine whether and how the temporal pattern of abundance was skewed. Due to computation restrictions of the program *moments*, the D'Agostino skewness test was not conducted in *Gomphus lucasii* due to the large number of records.

Results

Species richness and new records

A total of 42 species were recorded between 2010 and 2015, of which 18 (42.86%) were zygopterans and 24 (57.14%) anisopterans. Seven species had not been recorded before in the watershed in previously published works, namely *Aeshna affinis*, *Aeshna isosceles*, *Boyeria irene*, *Brachythemis impartita*, *Orthetrum trinacria*, *Coenagrion scitulum* and *Enallagma deserti*. Of the latter species, *Aeshna affinis*, *Aeshna isosceles*, *Brachythemis impartita*, and *Orthetrum trinacria* did not show signs of reproduction. However, the three other species were recorded as reproducers with larval records for *Boyeria irene* and exuviae and reproductive pairs for *Coenagrion scitulum* and *Enallagma deserti*. Over the six years of study, we assigned six species as apparently vagrant in the Seybouse watershed (*Onychogomphus uncatus*, *Aeshna affinis*, *Aeshna isosceles*, *Brachythemis impartita*, *Orthetrum trinacria*, and *Trithemis kirbyi*), while all the other 36 species are certain reproducers in at least one locality.

A list of species with localities where the species was recorded as reproducing or not is presented in Table S2. Across species, the mean number of localities was 10.95 ± 8.13 with 7.61 ± 8.02 ($63.03 \pm 30.68\%$) supporting reproductive populations (Table 1). A mean of

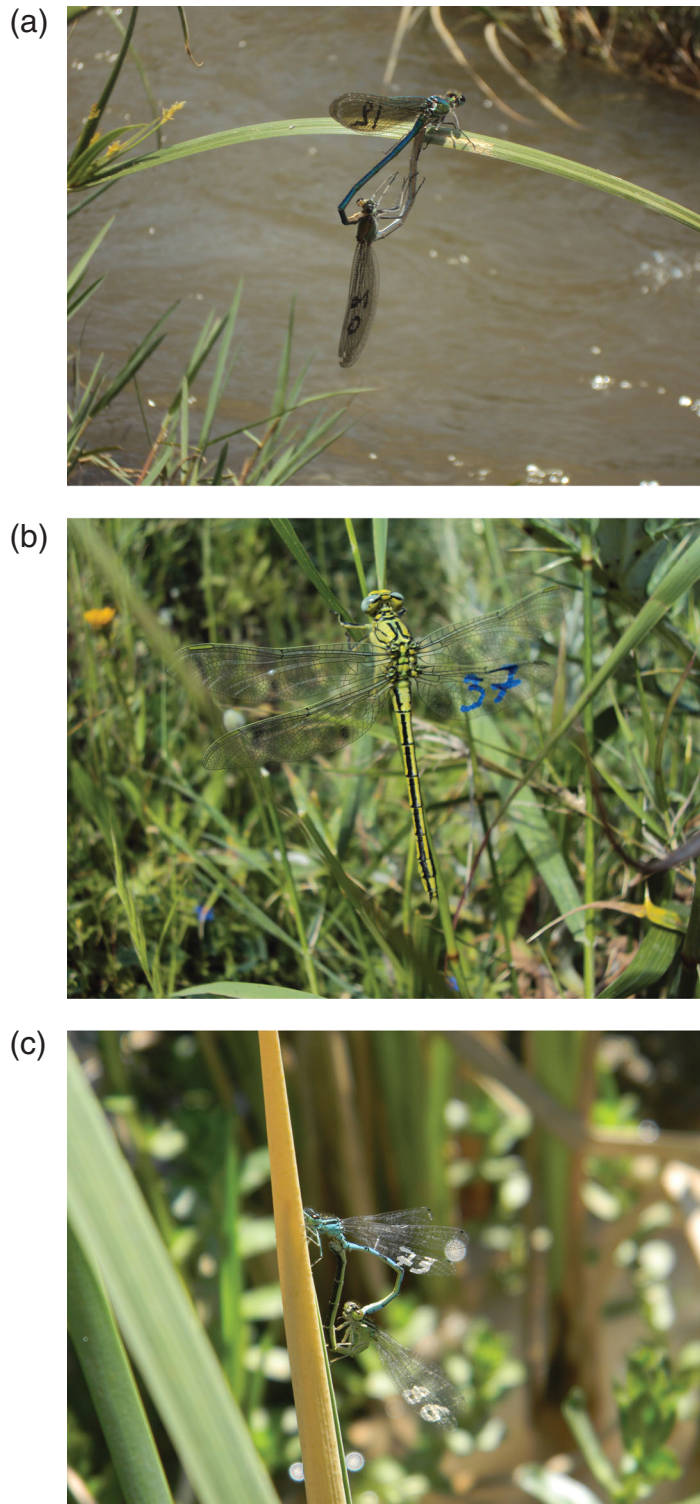


Figure 2. Marked adults in the field. (a) reproductive pair of *Calopteryx exul*; (b) juvenile male of *Gomphus lucasii*; (c) reproductive pair of *Coenagrion mercuriale*.

Table 1. Number of localities where species of the Seybouse watershed were observed.

Species ^a	Number of localities	New localities	Number of reproductive localities	Percentage of reproductive localities ^b
<i>Calopteryx exul</i>	12	6	10	83.33
<i>Calopteryx haemorrhoidalis</i>	21	12	13	61.90
<i>Ceragrion tenellum</i>	12	5	2	16.67
<i>Coenagrion caerulescens</i>	13	3	8	61.54
<i>Coenagrion mercuriale</i>	12	10	9	75.00
<i>Coenagrion puella kocheri</i>	10	8	4	40.00
<i>Coenagrion scitulum</i>	2	2	2	100
<i>Enallagma deserti</i>	7	7	6	85.71
<i>Erythromma lindenii</i>	14	8	13	92.86
<i>Erythromma viridulum</i>	4	3	4	100
<i>Ischnura graellsii</i>	40	22	40	100
<i>Ischnura pumilio</i>	6	4	3	50.00
<i>Lestes barbarus</i>	10	7	6	60.00
<i>Lestes numidicus</i>	9	8	4	44.44
<i>Lestes virens virens</i>	12	8	5	41.67
<i>Chalcolestes viridis</i>	13	5	6	46.15
<i>Platycnemis subdilatata</i>	31	18	31	100
<i>Sympecma fusca</i>	9	7	7	77.78
<i>Aeshna mixta</i>	12	9	5	41.67
<i>Aeshna affinis</i>	1	1	1	100
<i>Aeshna isosceles</i>	1	1	1	100
<i>Anax imperator</i>	25	15	18	72.00
<i>Anax parthenope</i>	11	9	8	72.73
<i>Boyeria irene</i>	3	3	3	100
<i>Crocothemis erythraea</i>	24	8	22	91.67
<i>Diplacodes lefebvrii</i>	9	5	6	66.67
<i>Onychogomphus costae</i>	11	8	8	72.73
<i>Onychogomphus forcipatus unguiculatus</i>	4	4	1	25.00
<i>Onychogomphus uncatus</i>	1	0	0	0.00
<i>Gomphus lucasii</i>	14	8	12	85.71
<i>Brachythemis impartita</i>	2	2	0	0.00
<i>Orthetrum coerulescens anceps</i>	16	7	13	81.25
<i>Orthetrum cancellatum</i>	16	12	9	56.25
<i>Orthetrum chrysostigma</i>	15	5	10	66.67
<i>Orthetrum nitidinerve</i>	15	7	6	40.00
<i>Orthetrum trinacria</i>	4	4	0	0.00
<i>Sympetrum fonscolombii</i>	12	6	6	50.00
<i>Sympetrum meridionale</i>	3	2	2	66.67
<i>Sympetrum striolatum</i>	8	4	8	100
<i>Trithemis annulata</i>	8	2	6	75.00
<i>Trithemis arteriosa</i>	5	2	2	40.00
<i>Trithemis kirby</i>	4	1	0	0.00

^aSpecies in bold are the study species that were used for population estimation.^bPercentage of reproductive localities is in respect to all localities where the species was observed

6.33 \pm 4.52 ($N = 42$) new localities were noted which represent 64.83 \pm 25.62% ($N = 42$) of all localities (Table 1). Of the 42 species, five (*Gomphus lucasii*, *Calopteryx exul*, *Lestes numidicus*, *Enallagma deserti* and *Platycnemis subdilatata*) are endemic to the Maghreb and two (*Orthetrum nitidinerve* and *Coenagrion caerulescens*) to the Mediterranean basin. All the seven latter species reproduce successfully in the Seybouse watershed.

Regarding the three threatened species *Calopteryx exul*, *Coenagrion mercuriale*, and *Gomphus lucasii*, six, 10, and eight new localities were recorded, respectively. In total, 12 localities were recorded for *C. exul*, 10 of which certainly supported reproducing populations. Of these 10 populations, two are now extinct. *C. mercuriale* was recorded in 12 localities, six of which are reproductive. Finally, *G. lucasii* was observed in 14 localities of which certain reproduction was

noted in 12 of them. The emergence of the latter lotic species was recorded in a pond (locality 34), but oviposition was not observed there.

Population estimation

The number of adults of *Calopteryx exul* marked during the flight season was 1417 (666 males and 751 females). The number of individuals of *Calopteryx exul* estimated by the POPAN model was 970.64 (95% CI: 929.0–1012.2) for females and 1237.19 (95% CI: 1140.1–1334.2) for males, giving a significant male biased sex ratio of 56.03% ($\chi^2 = 32.18$, $p < 0.0001$). The temporal pattern of abundance showed a significant positive skew (skewness = 0.483; D'Agostino skewness test: $z = 12.338$, $p < 0.0001$). The abundance peaked on day 16, which was also the day when half of the total abundance was recorded (Figure 3a).

A total of 1008 (772 males and 236 females) adults of *Coenagrion mercuriale* were marked and surveyed. The estimated total abundance of *Coenagrion mercuriale* was 412.9 (95% CI: 372.6–453.2) for females and 1352 (95% CI: 1254.6–1449.3) for males. The sex ratio was highly male biased (76.60%; $\chi^2 = 499.65$, $p < 0.0001$). The temporal pattern of abundance was slightly negatively skewed (skewness = -0.604; D'Agostino skewness test: $z = -11.646$, $p < 0.0001$), reaching 50% of its total size in 16 days and peaking on day 20 in both sexes (Figure 3b).

We marked 1316 individuals (870 males and 446 females) of *Gomphus lucasii*. The number of males was estimated as 7677.6 (95% CI: 5661.01–9694.19) and that of females as 3526.2 (95% CI: 2147.5–4904.8), showing a significantly male-biased sex ratio of 68.52% ($\chi^2 = 1538.3$, $p < 0.0001$). The number of males peaked on days 23 to 27 while that of females reached its highest number in day 12 (Figure 3c). The temporal pattern of abundance did not show significant skew (skewness = 0.058).

Discussion

The current paper presents estimations of population size of *Calopteryx exul*, *Coenagrion mercuriale* and *Gomphus lucasii* in natural populations in the Seybouse watershed, showing that their abundance is surprisingly large. In addition, the update of the local odonotofauna and the distribution of species based not only on adults but also on larvae and exuviae confirmed the reproductive state of species and highlighted the occurrence of new localities and new species within the watershed.

The number of species recorded in the Seybouse watershed represents 93.3% of the total diversity of Numidian (Samraoui & Corbet, 2000) and 25.45% of the Mediterranean odonotofauna (Riservato et al., 2009). This watershed also presented a relatively high degree of endemism with 16.67% of species endemic to the Mediterranean and 11.9% to the Maghreb. Besides the high species richness, a few threatened species have large distribution in this site. For example, compared to the localities presented in Khelifa et al. (2011) and Khelifa (2013), the number of localities where *Calopteryx exul* was recorded has doubled, with six new reproductive localities. According to the IUCN, the species has been recorded only in 32 localities in the Maghreb and many of the populations are extinct (Boudot, 2010a). Given the small geographic area of the Seybouse watershed, the 12 known localities of *C. exul* make the site of primary importance within its entire global range. Furthermore, *Coenagrion mercuriale* is regionally as threatened as the latter species with seven known localities in Algeria (Boudot et al., 2009). Within the Seybouse watershed, two localities were recorded in Khelifa et al. (2011) and one in Mahdjoub et al. (2014). However, our results present eight new localities with evidence of reproduction in all of

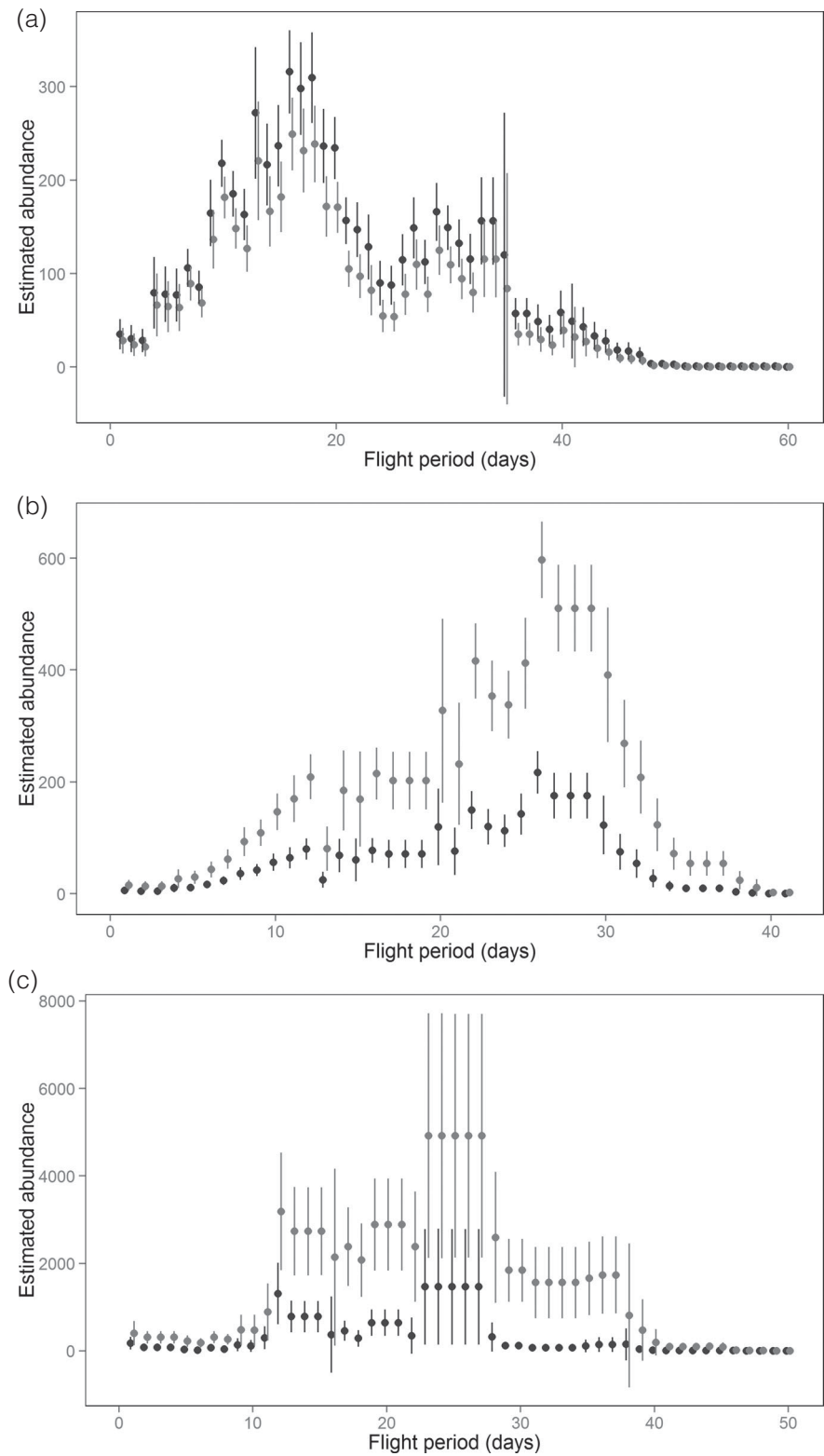


Figure 3. Temporal pattern of estimated abundance using the POPAN model. (a) *Calopteryx exul*; (b) *Coenagrion mercuriale*; (c) *Gomphus lucasii*. Black and gray colors refer to female and male, respectively. Error bars are 95% confidence intervals.

them. Thus, there are more localities in the Seybouse watershed than the remainder of the entire country. Likewise, given that only one population in Tunisia still exists, the Seybouse watershed harbors more populations than the latter country and 41.6% of Moroccan localities (Ferreira et al., 2015). In addition, unlike Moroccan populations there are populations at low, middle and relatively high elevation (Ferreira et al., 2015). *Gomphus lucasii* seems widely distributed in the Seybouse (13 localities) compared to old Numidian records presented by Samraoui and Corbet (2000) who stated that only three localities were likely to exist and 13 have become extinct over the last century. The recorded emergence of the species in a pond may be explained by the occurrence of a stream that flows into the pond, thus we are confident that the eggs were laid in the stream then, at some point of their larval life (probably later stadia), individuals entered the pond and carried out the ecdysis in the local bank vegetation. Therefore, we exclude the possibility that the species reproduces and survives as larvae in lentic water, as is commonly observed in its congeneric *G. vulgatissimus* (Dijkstra & Lewington, 2007).

The estimation of population size of the three studied populations of *Calopteryx exul*, *Coenagrion mercuriale* and *Gomphus lucasii* based on CMR showed astonishingly larger numbers compared to the IUCN estimations. In *Calopteryx exul*, the global population is estimated to be less than 10,000 adults (Boudot, 2010a) which is only 4.52 times larger than the Seybouse population, estimated as 2208 individuals. Considering that the latter estimate comes from one specific area that includes four localities, the population size of the Seybouse watershed, which encompasses nine reproductive localities is most likely as large as the global population presented by the IUCN. In the same way, the global population of *G. lucasii* presented by the IUCN is 2500 (Boudot, 2010b) which is obviously an underestimation because our study population is about 4.5 times larger. The claim that no population reaches 250 adults (Boudot, 2010b) is also erroneous because more than 1300 individuals were marked within a 2 km river stretch and around 1250 exuviae were collected within four 20-m stretches. No population estimation is given for the North African population of *C. mercuriale* by the IUCN. However, our study estimates a total of 1765 in one locality. We do not claim that all populations recorded in the Seybouse watershed harbors as large populations as the studied ones since we targeted what we expected to be the largest populations of the three species of the watershed.

The three species had different temporal patterns of abundance of adults, which is well explained by their different seasonal regulations. The IM50 was longest in *Coenagrion mercuriale*, at 20 days, similar to the number of days when half of the annual population emerges (EM50 = 23 days) reported by Mahdjoub, Khelifa, Zebba, Bouslama, and Houhamdi (2015) at the same site and during the same year. *Calopteryx exul* showed a shorter IM50 (16 days) which was close to the EM50 estimated using teneral (15 days) at the same population (Khelifa R., unpublished data). The shortest IM50 (12 days) was recorded in *Gomphus lucasii*, most likely because the dragonfly is a 'spring' species with an EM50 of nine to 10 days recorded in the studied population (Zebba, Khelifa, & Kahalerras, 2014).

The sex ratio of the three species was male biased. *Coenagrion mercuriale* and *Gomphus lucasii* presented a more biased sex ratio than *Calopteryx exul* and this is probably related to the ecological habits of the females. In the former species, females spend most of their time in terrestrial habitats and head to the water only to lay eggs (Mahdjoub et al., 2014; Zebba et al., 2014). Therefore, adult sampling next to the water often provides a male biased sex ratio (Stoks, 2001). On the other hand, *C. exul* females do not show sexual ecological segregation during the flight period. They forage far from the reproductive territories but next to the water and do not go further than a few meters from the watercourse towards terrestrial habitat (Khelifa unpublished data). Thus, the small male biased sex ratio might be related to differential survival probabilities between sexes. In fact, the main predators that were recorded in the study site were frogs. *C. exul* females perform non-contact oviposition and are closer to the water than the guarding male who is usually perched nearby. As a consequence, females are more exposed to frog predation

than males, which biases the mortality between sexes. The opposite pattern was recorded in *Calopteryx haemorrhoidalis* in a population whose dominant predators were spiders (Rehfeldt, 1992).

Although it appears that the present study shows good news about the status and distribution of three threatened species, the fate of these populations is far from peaceful. Several populations are undergoing high anthropogenic pressure including water pumping for agricultural purposes, artificialization of the watercourse bed, degradation of bank vegetation and pollution of water mainly with pesticides and organic compounds. Two reproductive populations of *Calopteryx exul* were recorded extinct from 2011 and three others (El Fedjoudj G, H and P) decreased abruptly during the past three years. Similarly, the habitat of *Coenagrion mercuriale* at the studied locality (Old Bridge) has been degraded recently with probable consequences on population size. An urgent management plan is needed to create protection areas to decrease the anthropogenic effects on some targeted natural populations in order to ensure the persistence of species of conservation concern in the Seybouse watershed.

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